Improved host-range determination in classical biological control of weeds

Dana Berner

Foreign Disease-Weed Science Research Unit, USDA, ARS

Ft. Detrick, MD

Mission of Foreign Disease - Weed Science Research Unit

- Find and develop foreign plant pathogens for <u>classical</u> biological control of introduced invasive weeds in the U.S.

Steps in Developing Plant Pathogens for Classical Biological Control of Weeds

- Find diseases of introduced weeds in the native (foreign) ranges of the weeds

- Isolate the pathogens

- Test the pathogens for damage

- Test the pathogens for their host range (safety)

Target:

▶ **Russian thistle or tumbleweed (*Salsola tragus*)**

Pathogens:

▶ *Colletotrichum gloeosporioides* f. sp. *salsolae* (CGS)
    Facultative parasitic fungus
    Hungary (other isolates from Greece and Russia)

▶ *Uromyces salsolae*
    Obligate parasitic rust fungus
    Russia

Tumbleweed/Russian Thistle

Colletotrichum gloeosporioides f. sp. *salsolae* (CGS)

Uromyces salsolae

Problem

How to best evaluate disease reaction of non-target plant species?

- Avoid discarding potentially beneficial organisms

- Ensure pathogen safety

Partial Disease Severity Results (CGS)

Table. Plants in the Chenopodiaceae

Genus	Species	No. Plants		Average Disease Rating
		Inoc	Pos	
Grayia	spinosa	10	0	0
Kochia	scoparia	15	0	0
Nitrophila	occidentalis	7	7	1.8
Salicornia	bigelovii	1	1	4.0
Salicornia	maritima	5	5	3.6
Salicornia	virginica	5	0	0
Salsola	kali	11	9	2.4
Salsola	orientalis	13	2	0.4
Salsola	paulsenii	5	5	4.0
Salsola	tragus	261	252	2.9
Salsola	australis	65	55	1.1
Suaeda	californica	1	0	0
Suaeda	taxifolia	4	4	2.2

NEED

- Environmentally-independent measure of disease severity

- Disease severity for <u>species</u> (vs. individual plants)

- An objective indicator of susceptible & non-susceptible <u>species</u>

- A means to evaluate rare and difficult or impossible to grow species

- A means to objectively compare disease on target vs. non-targets

Partial Solution
(next best)

- **Use ranks of disease severity ratings**
- **Use logit values of disease incidence**
- **Analyze using weighted mixed model ANOVA**
- **Produce least squares mean estimates**

Full Solution

Combine disease evaluation data with another statistical approach

- Incorporate <u>all available knowledge</u>
- Incorporate genetic relationships among species with variances and disease evaluation data
- Get broad-based predictions for <u>species</u>

Mixed Model Equations (MME) and Best Linear Unbiased Predictors (BLUPs)

- **Long-established quantitative genetics approach**

- Standard in generation of breeding values (BLUPs) for dairy cattle worldwide.
  - C. R. Henderson – 30+ years of publications from 1949

- Also for quarter horses, swine, trees, crop plants,……………

- Common objective:
  - **<u>Predict</u> breeding values (genetic merit of potential parents) -BLUPs**

BLUPs

Best – minimum mean squared error

Linear – linear function of the data

Unbiased – average value of the estimate equals average value of quantity being estimated; no estimable function bias

Predictor – realized value of a random variable

BLUPs are also parametric Bayes estimates

Value of the MME & BLUPs in Host Range Determination

- Predict susceptibility of plant <u>species</u> relative to that of the target species
- Use all available information
  - disease ratings or incidence
  - variance/covariance structure
  - genetic relationships among species
- Predict disease reactions for species that cannot be tested or only inadequately tested
- Determine the complete host-range of a pathogen among tested, inadequately tested, and not tested species
- Determine relevant lists of non-target species to test

How?

- Incorporate genetic relationships

- Incorporate genetic variances

- Incorporate performance (disease evaluation) data

How?

Incorporate genetic relationships from:

- Pedigrees and coefficients of co-ancestry

OR

- DNA sequences and genetic distance matrices

How?

Incorporate genetic variances from

- Heritability estimates

OR

- Estimate variance from data

How?

Incorporate performance data
(disease/damage evaluation data)

How?

- Integrate data into Mixed Model Equations

- Generate Best Linear Unbiased Predictors (BLUPs) for species

How?

- Generate DNA sequences, ITS (and/or other), for plant species

- Generate distance matrix among species based on these sequences

- Integrate these genetic distances with performance data

- Run the MME to generate BLUPs

Model
(in matrix notation)

- $y = XW + ZU + E$
  - y = n × 1 vector of ranks of disease ratings
  - X = n × 1 design vector of "1"s for the fixed intercept only
  - W = 1 × 1 unknown vector for the fixed effect parameters, in this case only the intercept
  - Z = n × j design matrix for the random effects, in this case species
  - U = j × 1 unknown vector of the random effects parameters
  - E = n × 1 vector of residuals (errors)

- variance of $y = S = ZGZ' + R$
  - G = j×j matrix of variances and covariances from distance matrix of DNA sequences and variance among species
  - R = n×n error matrix with known error variance as estimated from data

- $\hat{U} = GZ'\hat{S}^{-1}(y - X\hat{W}) = \underline{BLUPs}$

Partial G matrix

relationship matrix=(1-each element of distance matrix)

G= Variance among species × each element of relationship matrix

The Mixed Procedure

Estimated G Matrix

Row	Effect	taxon	Col1	Col2	Col3	Col4	Col5	Col6
1	taxon	Allenroloccident	2324.43	2173.27	732.64	1617.11	1617.11	1626.61
2	taxon	Allenrolvaginata	2173.27	2324.43	797.44	1764.68	1764.68	1777.79
3	taxon	Allium cepa	732.64	797.44	2324.43	650.33	643.54	656.05
4	taxon	Amaranthcaudatus	1617.11	1764.68	650.33	2324.43	2324.43	2309.55
5	taxon	Amaranthhypochon	1617.11	1764.68	643.54	2324.43	2324.43	2309.55
6	taxon	Amaranthretrofle	1626.61	1777.79	656.05	2309.55	2309.55	2324.43
7	taxon	Amaranthspinosus	1606.60	1757.78	649.26	2252.49	2252.49	2234.94
8	taxon	Aphanismblitoide	1653.06	1845.36	628.13	1729.70	1725.17	1743.58
9	taxon	Atriplexcanescen	1773.08	1850.73	753.39	1688.16	1688.16	1714.27
10	taxon	Atriplexlentifor	1763.55	1832.70	739.17	1702.23	1702.23	1727.96
11	taxon	Atriplexpatula	1776.28	1802.67	732.08	1743.21	1743.21	1752.92
12	taxon	Atriplexsemibacc	1746.04	1789.23	755.56	1674.71	1674.71	1701.34
13	taxon	Bassia hyssopif	1738.95	1811.29	681.80	1519.57	1519.57	1534.22
14	taxon	Betavulgaris	1678.15	1873.30	770.69	1756.55	1751.99	1765.33
15	taxon	Bougainvsp	1448.21	1487.43	777.71	1299.52	1299.52	1317.56
16	taxon	Brassicaoleracea	907.18	1274.46	490.90	833.56	825.87	855.44
17	taxon	Calendulofficina	1097.90	1227.04	538.18	862.29	855.95	863.39
18	taxon	Carduus acanthoi	1094.11	1172.19	542.17	1011.59	1005.48	995.09
19	taxon	Carduus pycnocep	1175.63	1269.35	511.21	1045.76	1039.69	1045.04
20	taxon	Carthamutinctori	1124.93	1172.05	473.60	1003.39	997.32	992.69
21	taxon	Centaurecyanus	1192.36	1340.50	594.01	1052.29	1046.16	1048.57
22	taxon	Centaurediffusa	1205.73	1332.94	564.33	1067.38	1061.29	1063.75
23	taxon	Centauresolstiti	1176.07	1290.59	555.17	1046.30	1040.16	1042.58
24	taxon	Chenopodalbum	1704.32	1783.93	879.26	2029.81	2029.81	2040.97
25	taxon	Chenopodambrosio	1520.15	1615.04	575.74	1435.99	1435.99	1469.92
26	taxon	Corisperpacificu	1711.90	1811.80	733.29	1609.67	1609.67	1619.01
27	taxon	Crupina	1112.26	1225.30	563.60	986.84	980.61	982.89
28	taxon	Cupressuabramsia	656.58	1090.99	131.31	573.32	579.02	573.09
29	taxon	Cupressugovgov	776.20	1307.58	361.01	645.84	651.49	645.10
30	taxon	Cynara scolymus	1199.24	1266.44	486.81	953.18	947.04	943.28
31	taxon	Daucus carota	1298.19	1372.51	750.74	1066.08	1066.08	1068.98
32	taxon	Endolepicovillei	1106.36	1185.95	547.26	949.13	942.91	978.72
33	taxon	Froelichgracilis	1558.34	1688.56	835.68	1494.70	1494.70	1478.55
34	taxon	Gossypiubarbaden	1050.18	1252.47	374.77	883.19	876.89	885.63
35	taxon	Halocnemstrobila	2098.08	2016.84	687.85	1641.30	1642.86	1652.23
36	taxon	Halostaccaspica	2108.77	2101.45	941.95	1650.30	1650.30	1661.32
37	taxon	Halothamsubaphyl	1785.12	1844.81	861.18	1565.67	1565.67	1569.76
38	taxon	Haloxyloammodend	1611.25	1510.97	685.80	1359.91	1359.91	1363.72
39	taxon	Haloxylopersicum	1794.67	1891.44	832.73	1611.78	1611.78	1615.92
40	taxon	Howelliaaquatili	1300.45	1392.66	691.56	1264.47	1264.47	1280.48
41	taxon	Kalidiumfoliatum	2039.20	2021.77	647.47	1653.06	1653.06	1663.92
42	taxon	Kochia american	1716.50	1789.88	706.86	1487.36	1487.36	1487.70
43	taxon	Lycopersesculent	1266.23	1315.56	708.02	1208.47	1202.50	1204.52
44	taxon	Malaco fasci	1089.53	1249.71	375.88	989.21	983.21	991.83
45	taxon	Mirabilimultiflo	1341.54	1364.42	686.08	1231.53	1231.53	1224.95
46	taxon	Opuntia ficus	1538.33	1616.04	713.39	1405.09	1399.28	1410.7

Phylogram from ITS sequence data
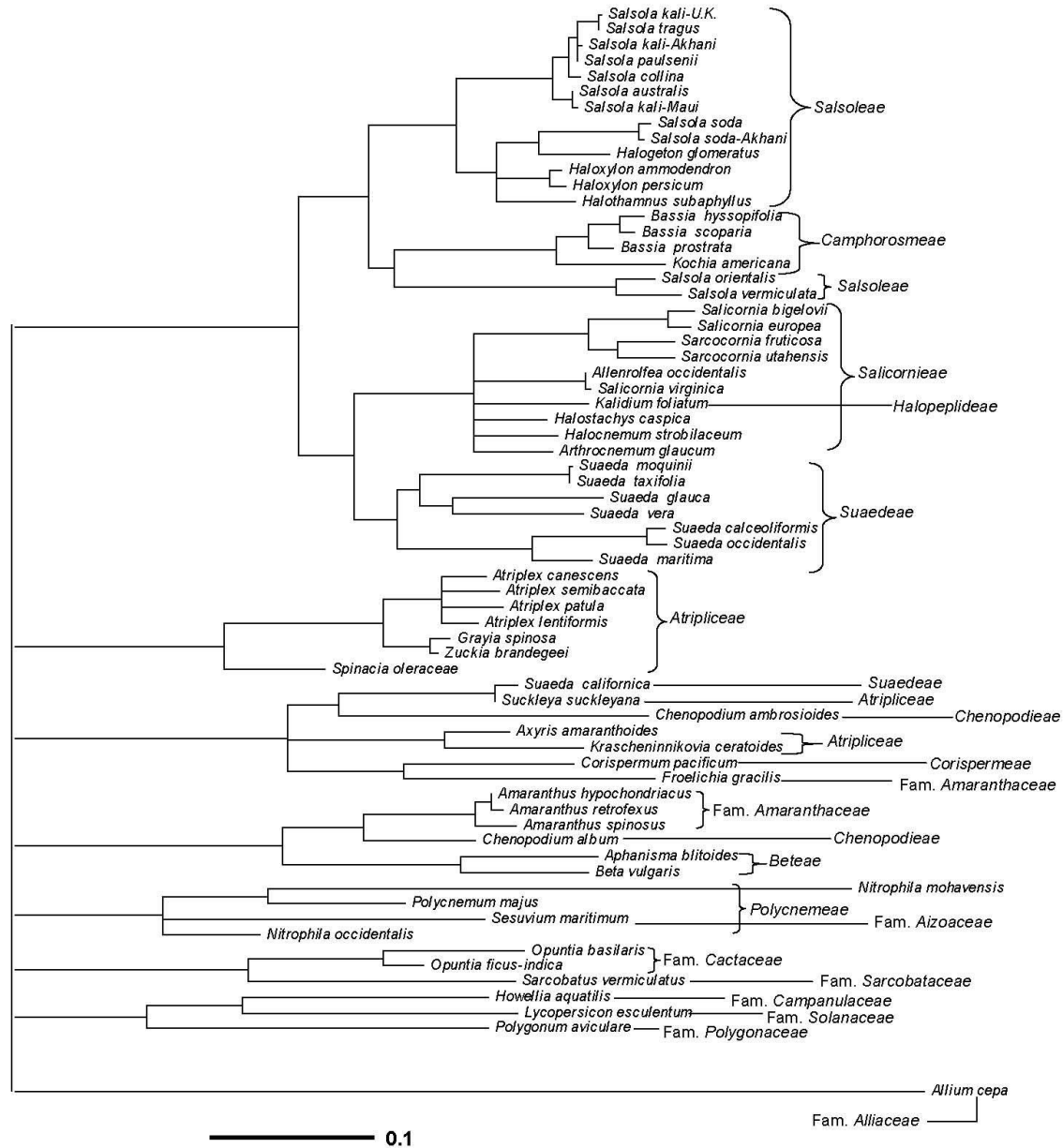
Power

- Beta (β) = probability of committing a Type II error and not rejecting a false null hypothesis
- Declaring no significant difference from zero when a difference exists
- Declaring a species not susceptible when it probably is
- Power = 1-β, probability of correctly rejecting a false null hypothesis
- Power values $\geq$ 0.80 are generally regarded as significant

Least squares means

Genus species	CGS				Uromyces salsolae			
	Least squares means estimates	Standard error of estimate	Pr>|t|	Power (1-β)	Least squares means estimates	Standard error of estimate	Pr>|t|	Power (1-β)
Salsola kali-U.K.	285.40	41.47	<0.0001	0.167	132.10	10.89	<0.0001	0.082
Salsola tragus	277.82	37.46	<0.0001	0.202	141.59	3.71	<0.0001	0.132
Salsola collina	285.98	35.00	<0.0001	0.235	62.05	0.54	NS	0.781
Salsola paulsenii	296.40	34.19	<0.0001	0.248	120.34	44.09	NS	<0.050
Salsola kali-Akhani	NT	NT	---------	------	NT	NT	---------	------
Salicornia bigelovii	247.06	17.30	<0.0001	0.999	61.21	2.22	NS	0.321
Salsola australis	111.24	12.72	NS	0.996	60.20	3.38	NS	0.200
Salsola kali-Maui	111.08	18.00	NS	0.884	36.67	52.20	NS	<0.050
Salicornia europaea	144.18	16.09	NS	0.922	60.03	4.76	NS	0.148
Sarcocornia fruticosa	NT	NT	---------	------	NT	NT	---------	------
Bassia hyssopifolia	239.08	29.50	<0.0001	0.355	52.64	39.61	NS	<0.050
Bassia scoparia	110.60	10.35	NS	>0.999	NT	NT	---------	------
Nitrophila occidentalis	254.37	81.79	NS	<0.100	60.20	3.38	NS	0.200
Halothamnus subaphyllus	NT	NT	---------	------	NT	NT	---------	------

59 species evaluated

6 susceptible (non-zero) species

CV= 3.31 to 407.87%

46 species evaluated

3 susceptible (non-zero) species

CV= 2.6 to 142.4%

BLUPs

Genus species	CGS				Uromyces salsolae			
	BLUP	Standard error of prediction	Pr>|t|	Power (1-β)	BLUP	Standard error of prediction	Pr>|t|	Power (1-β)
Salsola kali-U.K.	247.92	10.23	<0.0001	>0.999	102.94	5.30	0.006	>0.999
Salsola tragus	246.73	9.84	<0.0001	>0.999	101.97	5.10	0.007	>0.999
Salsola collina	235.40	10.70	<0.0001	>0.999	95.61	5.33	0.019	0.995
Salsola paulsenii	225.51	7.59	<0.0001	>0.999	96.90	4.65	0.023	>0.999
Salsola kali-Akhani	224.75	7.93	<0.0001	>0.999	97.02	4.85	0.023	>0.999
Salicornia bigelovii	213.39	14.95	<0.0001	>0.999	54.47	7.93	NS	>0.999
Salsola australis	208.14	10.29	<0.0001	>0.999	95.47	4.94	0.028	>0.999
Salsola kali-Maui	207.53	10.58	0.0001	>0.999	92.71	6.23	0.037	>0.999
Salicornia europaea	205.02	14.86	0.0002	0.996	54.55	7.93	NS	>0.999
Sarcocornia fruticosa	190.04	21.67	0.0027	0.978	55.43	9.21	NS	0.959
Bassia hyssopifolia	188.26	17.62	0.0026	0.960	67.32	9.44	NS	0.983
Bassia scoparia	187.80	9.01	0.0036	>0.999	68.09	9.72	NS	0.923
Nitrophila occidentalis	184.10	16.80	0.0070	>0.999	-------	-------	------	------
Halothamnus subaphyllus	176.36	18.05	0.0152	>0.999	82.58	8.10	NS	0.995

89 species evaluated

30 susceptible (non-zero) species

CV= 3.53 to 43.32%

66 species evaluated

7 susceptible (non-zero) species; all *Salsola* spp.

CV= 4.99 to 36.74%

BLUPs and the binary/binomial case

- Data converted to "1" or "0"

- Disease vs no disease

- Successes/attempts = x, e.g., 0.10, 0.20, 0.90, etc.

- Proportions of 0 and 1 set to 0.01 and 0.99, respectively.

- Logit transformation: $\log(x/1-x)$ or $\log(odds)$

Odds ratio

- Odds ratio= the natural logarithm to the power of the BLUP for each species, i.e., e^{BLUP} for logit values

- A probability of whether disease is likely

- Odds ratios > 1 indicate disease occurrence is likely

- The larger the odds ratio the greater the likelihood of disease

BLUPs

Genus species[1]	CGS				Uromyces salsolae			
	BLUP (Logit)	Standard error of prediction	Pr>|t|	Odds ratio	BLUP (Logit)	Standard error of prediction	Pr>|t|	Odds ratio
Salsola kali- U.K.	12.11	2.51	<0.0001	79.12	8.49	2.51	0.002	9.19
Salsola tragus	12.00	2.51	<0.0001	70.67	8.31	2.51	0.002	7.49
Salsola collina	10.94	2.55	<0.0001	24.48	7.12	2.52	0.007	1.62
Salsola paulsenii	9.94	2.77	0.0005	8.98	7.22	2.70	0.011	2.71
Salsola kali- Akhani	9.85	2.77	0.0006	8.24	7.24	2.70	0.011	2.78
Salicornia bigelovii	10.53	2.68	0.0002	16.31	0.06	2.69	NS	0.01
Salsola australis	8.76	2.55	0.0009	2.78	6.82	2.71	0.016	1.64
Salsola kali- Maui	8.81	2.57	0.0009	2.92	6.46	2.61	0.017	0.78
Salicornia europaea	9.46	2.64	0.0005	5.56	-0.01	2.69	NS	0.01
Sarcocornia fruticosa	7.93	2.92	0.0078	1.21	0.39	2.80	NS	0.02
Bassia hyssopifolia	9.27	2.86	0.0016	4.61	2.72	2.81	NS	0.08
Bassia scoparia	8.99	2.95	0.0030	3.50	2.54	2.88	NS	0.07
Nitrophila occidentalis	8.09	3.08	0.0101	1.42	-------	-------	------	------
Halothamnus subaphyllus	6.80	3.18	0.0351	0.39	4.62	3.05	NS	0.39

89 species evaluated

26 species with significant disease incidence

15 species with odds ratios greater than 1 -5 native spp.

66 species evaluated

8 species with significant disease incidence

7 species with odds ratios greater than 1 -no native spp.

Why?

- The large number of inter-specific relationships place the disease reaction of each species in context, genetically and mathematically, with **all** species analyzed.

- A spider-web analogy is apt: in the case of CGS there are 89 × 89 = 7921 interconnected nodes in the G matrix, and a force, e.g., disease, applied to any node "pulls" the other nodes, and web, in that direction. Forces at other nodes "pull" in opposing directions.

- Or, in the case of CGS, BLUPs reflect 59 × 59 (species with data) ÷ 2 = 1,740 fractional replications based on the genetic inter-relationships among these species.

- BLUPs reflect the disease reactions of each species plus the disease reactions of **all** of the other inter-related species.

Using BLUPs to construct non-target test lists

Target →

Genus species	BLUP	S.E.
Rhaponticum repens	70.73	35.51
Arctium minus	-3.66	36.72
Callistephus chinensis	7.68	36.09
Centaurea montana	5.77	35.67
Plectocephalus rothrockii	0.79	36.05
Cichorium intybus	-3.93	36.15
Cirsium pitcheri	0.79	35.77
Crupina vulgaris	12.83	36.65
Erigeron rhizomatus	16.58	35.85
Helianthis eggertii	3.95	36.33
Helianthis schweini	4.34	36.33
Krigia montana	-11.46	36.09
Picnomon acarna	3.64	36.14
Saussurea alpine	4.80	36.74
Serratula coronata	14.06	36.54
Solidago shortii	11.46	36.06
Stokesia laevis	15.41	35.44
Carthamus tinctorius	15.86	36.06
Cynara scolymus	1.55	35.86
Plectocephalus americana	4.25	35.28
Carduus tenufloris	-6.45	35.75
Carduus thoermeri	-3.97	35.72
Centaurea calcitrapa	-11.42	35.79

▢ = no evaluation data

⬭ = suspect reaction

- Iteratively test, re-analyze, re-test until suspect reactions clarified
- Iteratively include sequences of more species in each test-and- analysis cycle

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- **BLUPs are environmentally independent**

Advantages continued

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- **BLUPs predict <u>species</u> performance (vs. averages from the material tested in a greenhouse)**

- **The mixed model equations and BLUPs can be used to construct test plant lists**

Further Implications

- Useable with any agent and any target
- Useable in *ex-post* analyses
- Useable with historical data
- Useable by anyone

Questions

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- Why doesn't there seem to be a standard <u>objective</u> criterion for evaluation of susceptibility/damage?

- Why isn't <u>probability</u> of susceptibility/damage that criterion?

Questions

- Is this <u>species</u> susceptible based on one pustule on one leaf of one plant in a greenhouse test?

Uromyces salsolae

- Probably not: BLUP=63.3; Pr>|t|=0.95; odds ratio=0.013
- Doubtful that even the <u>plant</u> is susceptible
- Initial APHIS feedback seems to disagree

Questions

• Whatever happened to the scientific method and rigorous hypothesis testing?

$$Ho: \mu_1 = \mu_2 = \mu_n = 0$$

• Is this not necessary in biological control of weeds?

• Or is science simply not necessary in risk (host range) evaluation?

Recent Publications

•Berner, D.K. 2010. BLUP, a new paradigm in host-range determination. Biological Control 53: 143-152.

•Berner, D. K., W. L. Bruckart, C. A. Cavin, J. L. Michael, M. L. Carter, and D. G. Luster. 2009. Best linear unbiased prediction of host range of the facultative parasite *Colletotrichum gloeosporioides* f. sp. *salsolae*, a potential biological control agent of Russian thistle. Biological Control 51:158-168.

•Berner, D. K., W. L. Bruckart, C. A. Cavin, and J. L. Michael. 2009. Mixed model analysis combining disease ratings and DNA sequences to determine host range of *Uromyces salsolae* for biological control of Russian thistle. Biological Control 49:68-76.

"Validation"?

• The MME and BLUPs are long-accepted science that do not need validating per-se
• Validate the approach with weed biological control agents?
  • How?
  • Compare to second best approach?
  • Just did that and demonstrated a more conservative safer approach, i.e., most valid approach
  • Generate BLUPs for released agents?
    • In progress
    • Guaranteed to be more predicted susceptible spp. than occur in the field
  • Generate BLUPs for released agents with non-target effects?
    • Looking for volunteer datasets
    • Anticipate accurate prediction of non-target effects
    • No such data for plant pathogens

If things go wrong – the legal locomotive

Pseudo-science decision making
(subjectivity)

Science-based decision making
(probability)

No biological control

Effective and safe biological control

Is "what you see" what you get?

- Simple random sampling of test material assumes that the samples are truly representative of the species

- False positives and negatives, from a species perspective, are a real probability

- No indication of probability of "correct" evaluation

Is "what you see" what you get?

- In the case of CGS, <u>each</u> BLUP in our evaluation is based on 89 species' interactions in addition to observed data

- This greatly increases the probability that the BLUPs are representative of the species

- Probabilities of taxon differences can be tested